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(54) POLYSACCHARIDE-PROTEIN CONJUGATES

KONJUGATE BESTEHEND AUS POLYSACCHARID UND PROTEIN

CONJUGUES DE POLYSACCHARIDE-PROTEINE

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Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates, in general, to polysaccharide-protein conjugates and vaccines. In particular the present invention relates to polysaccharide-protein conjugates that elicit serum IgG and IgM antibodies to both poly $\alpha(2\rightarrow8)$ NeuNAc and poly $\alpha(2\rightarrow9)$ NeuNAc.

Background Information

[0002] Neisseriae meningitidis are a major cause of systemic infections, especially meningitis, in humans. Capsular polysaccharide (CP) vaccines are licensed for meningococcal groups A,C,Y, and W135. Diseases caused by group B meningococci continue to occur in endemic and epidemic forms and remain an important health problem (Gotschlich, E.C. (1984) in Bacterial Vaccines. Ed. Germanier (Academic Press, NY) pp. 237-255; Peltola, H. (1983) Bev. Infect. Dis. 5, 71-91; Poolman, J.T. et al. (1986) Lancet, ii,555-557). Escherichia coli (E. Coli) K1 is a major cause of neonatal meningitis, upper urinary tract infections and systemic infections in hospitalized patients and in domesticated an laboratory animals (Robbins, J.B. et al. (1974) N. Eng. J. Med. 290, 1216-1220; Kaijser, B. et al. (1977) Lancet i, 663-664; Cross, A.S. et al. (1984) J. Infect. Dis. 149, 184-193; Orskov, I., & Orskov, F. (1985) J. Hyg. Camb. 95, 551-575). Despite antibiotic treatment and supportive care, meningitis caused by these two pathogens continues to exert a high morbidity, including permanent CNS injury, and mortality (Peltola, H. (1983) Rev. Infect. Dis. 5, 71-91; Schneerson, R. (1988) in Understanding Mental Retardation, ed, Kavanagh, J.F. (Paul Brookes Publishing Co. Baltimore), pp. 237-249; Brandtzaeg, P. et al. (1989) J. Infect, Dis. 159, 195-204; McCraken, G.H., Jr. et al. (1974) Lancet, ii, 246-250).

[0003] The CP of Group B meningococci and of E. coli K1 are identical (poly $\alpha(2\rightarrow8)$ NeuNAc) and serve as essential virulence factors and protective antigens for both pathogens (Grados, O., & Ewing, W.H. (1970) J. Infect. Dis. 122, 100-103; Kasper, D.L. et al. (1973) J. Immunol. 110, 262-268; Bhattacharjee, A.K. et al. (1975) J. Biol. Chem. 250, 1926-1932; Robbins, J.B. et al. (1974) N. Eng. J. Med. 290, 1216-1220). Poly $\alpha(2\rightarrow8)$ NeuNAc is also a surface antigen of Moraxella nonliquefaciens and Pasteurella haemolytica, serotype A-2 (Bøvre, K. et al. (1983) NIHP Annals. 6, 65-73; Devi, S.J.N. et al. (1991) Infect. Immun. 59, 732-736; Adlam, C. et al. (1987) FEMS Microbiol. Lett. 42, 23-25). The latter is the major cause of outbreaks of pasteurellosis in young lambs which suggests that poly $\alpha(2\rightarrow8)$ NeuNAc may serve as a virulence factor for yet another bacterial species.

[0004] Attempts to induce protective immunity to group B meningococci and E. coli K1 have been thwarted because poly $\alpha(2\rightarrow8)$ NeuNAc, alone or complexed to outer membrane proteins, induced low and transient levels of IgM antibodies (Kasper, D.L. et al. (1973) J. Immunol. 110, 262-268; Wyle, F.A. et al. (1972) J. Infect. Dis. 126, 514-522; Zollinger, W.D. et al. (1979) J. Clin. Invest. 63, 836-842; Moreno, C. et al. (1985) Infect. Immun. 47, 527-533; Frasch, C.E. et al. (1988) J. Infect. Dis. 158, 710-718; Lifely, M.R. et al. (1991) Vaccine 9, 60-66). Covalent attachment of periodate-treated (Jennings, H. & Lugowshi, C. (1981) J. Immunol. 127, 1011-1018) or acid-hydrolyzed poly $\alpha(2\rightarrow8)$ NeuNAc (Porro, M. et al. (1983) Med. Trop. 43, 129-132) to a protein also failed to elicit antibodies to this antigen. Further, this CP has been considered as a "self antigen" because $\alpha(2\rightarrow8)$ NeuNAc is found as monomers or dimers on glycoproteins and gangliosides in adults and up to $\approx$11 residues in fetal tissues including N-CAMSs (Finne, J. et al. (1983) Lancet, ii, 355-357; Finne, J. et al. (1987) J. Immunol. 138, 4402-4407; Soderstrom, T. et al. (1984) N. Eng. J. Med. 310, 726-727). Accordingly, investigators have studied other components, such as LPS, outer membrane proteins and iron-binding proteins, or chemically modified poly $\alpha(2\rightarrow8)$ NeuNAc, as potential vaccines (Zollinger, W.D. et al. (1979) J. Clin. Invest. 63, 836-842; Moreno, C. et al. (1985) Infect. Immun. 47, 527-533; Frasch, C.E. et al. (1988) J. Infect. Dis. 158, 710-718; Jennings, H.J. et al. (1984) Infect. Immun. 43, 407-412; Jennings, H.J. et al. (1986) J. Immunol. 137, 1708-1713; Frasch, C.E. (1989) Clin. Microbiol. Rev. 2(Suppl), S134-S138).

[0005] Most newborns and adults have bactericidal antibodies to the three major serogroups (A,B,C) of meningococci (Goldschneider, I. et al. (1969) J. Exp. Med. 129, 1307-1326); most of the bactericidal activity, including of group B meningococci, was removed by adsorption with the homologous CP (Frasch, C.E. et al. (1988) J. Infect. Dis. 158, 710-718; Brandt, B.L. et al. (1972) J. Immunol. 108, 913-920; Kasper, D.L. et al. (1973) J. Infect Dis. 127, 378-387; Skevakis, L. et al. (1984) J. Infect. Dis. 149, 387-396). The peak incidence of disease caused by meningococci, including group B, is when the maternally-derived antibodies have waned and the adult levels have not yet developed (Gotschlich, E.C. (1984) in Bacterial Vaccines. Ed. Germanier (Academic Press, NY) pp. 237-255; Goldschneider, I. et al. (1969) J. Exp. Med. 129, 1307-1326). Rises in poly $\alpha(2\rightarrow8)$ NeuNAc antibodies, including those of the IgG isotype, are detectable in patients convalescent from group B meningococcal meningitis (Wyle, F.A. et al. (1972) J. Infect. Dis. 126, 514-522; Zollinger, W.D. et al. (1979) J. Clin. Invest. 63, 836-842; Frasch, C.E. et al. (1988) J. Infect. Dis. 158, 710-718; Skevakis, L. et al. (1984) J. Infect. Dis. 149, 387-396; Craven, D.E. et al. (1982) Infect. Immun. 37, 132-137; Mandrell,

R.E. & Zollinger, W.D. (1982) J. Immunol. 129, 2172-2178; Leinonen, M. & Frasch, C.E. (1982) Infect. Immun. 38, 1203-1207). Polyclonal and monoclonal (mAb) poly α(2→8) NeuNAc antibodies were raised in animals by multiple intravenous injections of bacteria (Robbins, J.B. et al. (1974) N. Eng. J. Med. 290, 1216-1220; Moreno, C. et al. (1985) Infect. Immun. 47, 527-533; Mandrell, R.E. & Zollinger, W.D. (1982) J. Immunol. 129, 2172-2178; Allen, P.Z. et al. (1982) J. Clin. Microbiol. 15, 324-329; Craven, D.E. et al (1979) J. Clinc. Microbiol. 10, 302-307; Frosch, M. et al. (1985) Proc. Natl. Acad. Sci. (USA) 82, 1194-1198). Monoclonal antibodies to this antigen were identified in a healthy 81 year old male and from hybridoma cultures (Kabat, E.A. et al. (1986) J. Exp. Med. 164, 642-654; Kabat, E.A. et al. (1988) J. Exp. Med. 168, 699-711; Raff, H.V. et al. (1988) J. Infect. Dis. 157, 188-126). These antibodies exert biologic activities which have been correlated with protective immunity; 1) complement-dependent bacteriolysis on Group B meningococci (Gotschlich, E.C. (1984) in Bacterial Vaccines. Ed. Germanier (Academic Press, NY) pp. 237-255; Goldschneider, I. et al. (1969) J. Exp. Med. 129, 1307-1326); 2) protection against lethal infection of rodents by E. coli K1 (Robbins, J.B. et al. (1974) N. Eng. J. Med. 290, 1216-1220; Glode, M.P. et al. (1977) Infect. Immun. 16, 75-80; Kim, K.S. et al. (1985) Infect. Immun. 50, 734-737).

[0006] There are two other bacterial NeuNAc polymers: 1) group C N. meningitidis CP composed of poly α(2→9) NeuNAc; most strains are variably O-acetylated at C7 or C8 (Bhattacharjee, A.K. et al. (1975) J. Biol. Chem. 250, 1926-1932.) Although differing from poly α(2→8) NeuNAc only by linkage, poly α(2→9) NeuNAc is immunogenic and is a licensed vaccine against group C meningococci (World Health Organization Expert Committee on Biological Standardization.) (1977) Technical Report Series, 610. WHO, Geneva, Switzerland); 2) E. coli K92 CP (Figure 1) with the disaccharide repeat unit of alternating α(2→8), α(2→9) NeuNAc (The structure of this polysaccharide can be written as 9)-NeuNAc-α-(2→8)-NeuNAc-α-(2→.) (Robbins, J.B. et al. (1972) Infect. Immun. 6, 651-656; Glode, M.P. et al. J. Infect. Dis 135, 94-102; Egan, W. et al. (1977) Biochem. (USA) 16, 3687-3692; Glode, M.P. et al. (1979) J. Infect. Dis. 139, 52-59). Both group B and group C meningococcal antisera precipitate with E. coli K92 CP (Glode, M.P. et al. (1977) J. Infect. Dis. 135, 94-102; Egan, W. et al. (1977) Biochem. (USA) 16, 3687-3692; Glode, M.P. et al. (1979) J. Infect. Dis. 139, 52-59). Multiple i.v. injections of killed E. coli K92 bacteria induced precipitating antibodies to poly α (2→9) NeuNAc and to poly α(2→8),α(2→9) NeuNAc but not to poly α(2→8) NeuNAc (Glode, M. P. et al. (1977) J. Infect. Dis. 135-94-102). Injection of E. coli K92 CP induced poly α(2→9) NeuNAc antibodies in adult volunteers; antibodies to poly α(2→8) NeuNAc were not measured (Glode, M.P. et al. (1979) J. Infect. Dis. 139, 52-59).

[0007] US-A-4695624 discloses conjugates of a protein and a capsular polyanionic polysaccharide, capable of raising antibodies to both the protein and to the polysaccharide. The protein may be tetanus toxoid. The polysaccharide may be obtained from, *inter* alia, *N. meningiditis* type B, E. *coli* K1 or *E. coli* K92.

Summary of the Invention

[0008] According to one aspect of the present invention, a conjugate of *Escherichia coli* K92 capsular polysaccharide and a carrier protein is used for the manufacture of a medicament for use in the prevention of infection caused by non-K92 bacterial microorganisms having poly α(2→8)NeuNAc capsular polysaccharide surface antigens and by non-K92 bacterial microorganisms having poly α(2→9)NeuNAc capsular polysaccharide surface antigens. The conjugate elicits the production of antisera containing (i) antibodies reactive with the non-K92 bacterial microorganisms having poly α (2-8)NeuNAc capsular polysaccharide surface antigens; (ii) antibodies reactive with the non-K92 bacterial microorganisms having poly α(2-9)NeuNAc capsular polysaccharide surface antigens; and (iii) antibodies reactive with the carrier protein.

[0009] According to a second aspect of the present invention, antisera as defined above are used for the manufacture of a medicament for use in passive immunisation against infection by non-K92 bacterial microorganisms as defined above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Figure 1. Structure of the Escherichia coli K92 capsular polysaccharide: poly α(2→8),α(2'→9) NeuNAc (Egan, W., et al. (1977) Biochem. (USA) 13, 3687-3692).

[0011] Figure 2. Gel filtration of K92-TT (tetanus toxoid) conjugate. 1.0 ml of K92-TT, was passed through a column of 4B-CL Sepharose (2.5x90cm) in 0.2M NaCl. The fraction size vas 2.0 ml and the eluent was monitored by assay of NeuNAc (Yao, K. & Ubuka, T. (1987) Acta Med. Okayama. 41, 237-241) and by absorbance at 280 nm (World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland; Schneerson, R. et al. (1980) J. Exp. Med. 152, 361-376).

[0012] Figure 3. Double immunodiffusion with K92 conjugate: Center well - K92-TT, 0.1 mg/ml, Well A - rabbit antiserum to Escherichia coli K92 cells, Well B - mouse tetanus toxin antiserum.

DETAILED DESCRIPTION OF THE INVENTION

[0013] The present invention relates to the use of a polysaccharide-protein conjugate and a vaccine. This conjugate includes a polysaccharide and a carrier protein and is capable of eliciting serum IgG and IgM antibodies to both poly $\alpha(2\mapsto8)$ NeuNAc and poly $\alpha(2\mapsto9)$ NeuNAc) in a mammal or bird. The carrier is associated with the polysaccharide in such a way as to increase the immunogenicity of the polysaccharide and to confer upon it the properties of both eliciting a booster response and IgG antibodies. These immunologic properties should be elicited by the protein-polysaccharide vaccine alone. Addition of adjuvants, such as aluminium salts, bacterial murein structures in saline or in emulsions, may be helpful in eliciting or in enhancing the production of poly $\alpha(2\mapsto8)$ NeuNAc and poly $\alpha(2\mapsto9)$ NeuNAc Antibodies by the E. coli K92 conjugate vaccines. In one preferred embodiment, the carrier protein is covalently bound to the polysaccharide. The covalent bond should preserve the immunologic properties of the native polysaccharide and native protein. Some proteins that could serve as effective carriers for covalently bound polysaccharide-protein conjugates are albumins, pharmacologically active proteins that have been detoxified, by chemical or genetic mechanisms, including diphtheria, tetanus, pertussis, Pseudomonas aeruginosa exotoxin A and Stapylococcus aureus toxins, synthetic polypeptides, bacterial outer membrane proteins and viral proteins (Schneerson, R. et al. (1980) In: New Developments with Human and Veterinary Vaccines. Eds. Mizrahi et al., New York, Alan R. Liss; Schneerson, R. et al. (1987) In: Towards Better Carbohydrate Vaccines. Eds., Bell, R. & Torrigiani, G., World Health Organization, John Wiley & Sons, Ltd.). Carriers for the K92 NeuNAc polysaccharides should be proteins that are immunogenic and elicit booster responses by themselves. Carriers should have the necessary groups that allow the synthesis of conjugates with the E. coli K92 polysaccharides. Carriers should confer the properties of increased immunogenicity and booster responses to the E. coli K92 including the formation of both IgM and IgG antibodies to these polysaccharides (Schneerson et al (1987) In: Towards Better Carbohydrate Vaccines. Eds., Bell, R. Torrigiani, G., World Health Organization, John Wiley & Sons, Ltd.). In another preferred embodiment, the polysaccharide and protein are covalently bound by a linker. An effective linker has been found to be adipic acid dihydrazide. Other linkers could be diaminohexane, amino epsilon caproic acid, N-hydroxysuccinimide acid anhydride based heterobifunctional linkers as illustrated by N-succinimidyl 3-(2-pyridyldithio) priopionate (SPDP). Other cross-linking compounds can be used to synthesize the conjugate, provided they are not toxic and result in a conjugate that elicits poly $\alpha(2\mapsto8)$ NeuNAc and poly $\alpha(2\mapsto9)$ neuNAc antibodies (Robbins, J.B. Schneerson, R. (1990) J. Infect. Dis. 161:821-832). A linker is a molecule which may be used to covalently bind the polysaccharide to the protein. A chemical reaction with each end of the linker changes the structure of the linker. For example, after adipic acid dihydrazide chemically combines with the polysaccharide and the protein to form a conjugate, the polysaccharide and protein are bound by an adipic acid dihydrazido linkage. The polysaccharide comprises a heteropolymer of $\alpha(2\mapsto8),\alpha(2\mapsto9)$ NeuNAc, of the conjugate i.e., K92 capsular polysaccharide surface antigen. In yet another preferred embodiment, the carrier protein is tetanus toxoid. Additional carrier proteins that may be used include albumins (Schneerson, R., et al. (1980) J. Exp. Med. 152, 361-376), diphtheria toxoid (Schneerson, R., et al. (1980) J. Exp. Med. 152, 361-376), and Pseudomonas aeruginosa exotoxin A and mutants of this protein (Fattom A., et al. (1990) Infect. Immun. 58, 2367-2374).

[0014] In another embodiment, the present invention relates to a pharmaceutical composition comprising the above described polysaccharide-protein conjugate in an amount sufficient to prevent systemic infections including meningitis, caused by group B or group C Neisseria meningitidis, Escherichia coli K1, Moraxella nonliquefaciens, Pasteurella haemolytica, or other microorganisms containing poly $\alpha(2\mapsto8)$ NeuNAc, poly $\alpha(2\mapsto9)$ NeuNac, or poly $\alpha(2\mapsto8)$,$\alpha(2\mapsto9)$ NeuNAc, surface antigens and a pharmaceutically acceptable diluent, carrier, or excipient. The pharmaceutical composition of the invention includes polysaccharide conjugate in a quantity selected depending on the route of administration. Although subcutaneous or intramuscular routes of administration are preferred, the above described polysaccharide-protein conjugate could also be administered by an intraperitoneal or intravenous route. One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can be readily determined. Suitable amounts might be expected to fall within the range of 5.0 to 100.0 μg per dose of either the polysaccharide or the protein (The ratios of polysaccharide and protein that comprise the conjugate may differ. The dosages mentioned for each component are within the expected range.).

[0015] A method of preventing systemic infections caused by Groups A, B, and C Neisseria meningitidis in an animal, by administering to the animal the above-described polysaccharide-protein conjugate and a Group A meningococcal polysaccharide-protein conjugate under conditions such that the infections are prevented is described as well. The compositions also serve as vaccines.

[0016] The first step of a method for producing the conjugate comprises derivatizing the polysaccharide by using, for example, adipic acid dihydrazide in a carbodiimide reaction, or alternative agents/protocols. Adipic acid dihydrazide may be substituted in the carbodiimide reaction with other dihydrazide compounds or diamino compounds (for example: diamino hexane). Other derivatives of the polysaccharides could be made in order to covalently bind them to proteins. These include the use of disulfide bonds linked by heterobifunctional reagents (Szu, S.C., et al. (1986) Infect. Immun. 54, 448-455; Szu, S.C., et al. (1987) J. Exp. Med. 166, 1510-1524).

[0017] After derivatizing the polysaccharide, the next step of the method involves conjugating the derivative to a protein. Preferably, the adipic acid hydrazide derivative of the polysaccharide is conjugated to the protein by mixing the derivative with the carrier protein at equal concentrations and adjusting the pH to a pH in the range between 6.1 and 7.0. The reactants are dissolved in 0.2M NaCl and the temperature is at 3-8°C. Then, 1-ethyl-3(3-dimethylamino-propyl) carbodiimide (EDAC) is added to a final concentration less than 0.3M. The original pH is maintained for 3 hours. Next, the reaction mixture is dialyzed against 0,2M NaCl at 3-8°C for 3 days with multiple changes of the outer fluid. This synthetic scheme of multipoint attachment does not grossly fragment the poly $\alpha(2\rightarrow8)$ NeuNAc or poly $\alpha(2\rightarrow8)$, $\alpha(2\rightarrow9)$ NeuNAc and may provide conformational stability to the polysaccharide.

[0018] The invention is described in further detail in the following non-limiting examples.

EXAMPLES

[0019] The following protocols and experimental details are referenced in the examples that follow:

Bacteria. E. coli 07:K1:H- strain C94, E. coli 016:K1H:H-, stable in the 0 acetyl negative form (OAc), E. coli 075: K1:H-, OAc+, strain LH, (Lars A. Hanson, Goteborg, Sweden), E. coli 013:K92:H4, strain N67 have been described (Robbins, J.B. et al. (1972) Infect. Immuno. 6, 651-656). Group B meningococci, serotype 6, strain M990 and strain B11, and Group C meningococcus, strain C11, were provided by Carl E. Frasch, FDA, Bethesda, Maryland.

Polysaccharides and proteins. CP were purified from Group B meningococcus, strains B11 and M990, E. coli strains C94, LH, 016:K1:H- and N67 (World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland). These CP contained <1.0% of protein and nucleic acid, 75 to 87% NeuNAc (Yao, K. & Ubuk, T. (1987) Acta Med. Okayama. 41, 237-241), <0.01% of LPS and had Kd values through 4B-CL Sepharose of ~0.5 (World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland). The OAc contents were 1.62 µM/mg for LH and 1.39 µM/mg for the group C meningococcal CP (Bhattacharjee, A.K. et al. (1975) J. Biol. Chem. 250, 1926-1932; World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland). The ^{13}C and proton NMR spectra of the poly $\alpha(2\rightarrow8)$ NeuNAc and K92 CP were identical to those reported for these two polymers (Bhattacharjee, A.K. et al. (1975) J. Biol. Chem. 250, 1926-1932; Egan, W. et al. (1977) Biochem. (USA) 16, 3687-3692). Group C meningococcal CP was obtained from Pat McVerry, Connaught Laboratories Inc., Swiftwater, PA, and tetanus toxoid (TT), lot GYA, and group A meningococcal CP from Dominique Schulz, Pasteur Merieux Serums and Vaccines, Lyon, France. Type III, group B steptococcus CP was purified in the laboratory (Lagergard, T. et al. (1990) Infect. & Immun. 58, 687-694).

Hyperimmune sera. Antisera, prepared by intravenous injections of killed cells of Group B meningococci, strain B11 (horse 46), Group C meningococci, strain CII (burro 211) and rabbit E. coli K92 (Drs. Ida and Frits Orskov, Statens Seruminstitut, Copenhagen, Denmark) have been described (Orskov, I., & Orskov, F. (1985) J. Hyg. Camb. 95, 551-575; Allen, P.Z. et al. (1982) J. Clin. Microbiol. 15, 324-329; Golde, M.P. et al (1977) J. Infect. Dis. 135, 94-102; Orskov F. et al (1979) J. Exp. Med. 149, 669-685). Mice were injected with formalin-killed cells and their sera harvested as described (Orskov, I., & Orskov, F. (1985) J. Hyg. Camb. 95, 551-575; Lagergard, T. et al. (1990) Infect & Immun. 58, 687-694). Antisera for standards were produced in NIH general purpose mice by i.p. injection of 5.0 µg of either TT or K1-TT_1 in Freund's adjuvants (Lagergard, T. et al. (1990) Infect. & Immun. 58, 687-694).

Serology. Double immunodiffusion was performed in 0.6% agarose. ELISA was performed using biotinylated CP (Sutton, A. et al (1985) J. Immunol. Meth. 82, 215-224). Murine sera were assayed for poly $\alpha(2\rightarrow8)$ NeuNAc and poly $\alpha(2\rightarrow9)$ NeuNAc and TT antibodies using alkaline-phosphatase-labeled goat anti-murine immunoglobulins (Kirkgaard & Perry Inc., Gaithersburg, MD) (Lagergard, T. et al. (1989) Infect. & Immun. 58, 687-694; Sutton A. et al (1985) J. Immunol. Meth. 82, 215-224). Murine IgM mAb to poly $\alpha(2\rightarrow8)$ NeuNAc (Wendell Zollinger, Walter Reed Army Institute of Research, Washington, D.C.) and murine IgM and IgG mAb to poly $\alpha(2\rightarrow9)$ NeuNAc (Kathryn Stein, FDA, Rockville, MD) were used as reference standards (Mandrell, R.E. & Zollinger, W.D. (1982) J. Immunol. 129, 2172-2178; Rubinstein, L.J. & Stein, K.E. (1988) J. Immunol. 141, 4357-4362). Human poly $\alpha(2\rightarrow8)$ NeuNAc antibodies were assayed as described (Claesson, B.O. et al. (1988) J. Pediatr. 112, 695-702). A human IgM mAb (Elvin Kabat, Columbia University, NY) (Kabat, E.A. et al (1986) J. Exp. Med. 164, 642-654; Kabat E.A. et al. (1988) J. Exp. Med. 168, 669-711) and a high-tittered human sera (GH) were used as references for human poly $\alpha(2\rightarrow8)$ NeuNAc antibodies and the data are expressed as µg/ml for the IgM and as percent of the standard for IgG.

The effect of temperature upon IgG binding to poly $\alpha(2\rightarrow8)$ NeuNAc and poly $\alpha(2\rightarrow9)$ NeuNAc was assayed with sera from mice injected with bacteria or three times with 10.0 µg of conjugates. The data are expressed as the percent binding at 37°C compared to 22°C.

Synthesis of conjugates. It was confirmed that treatment at pH <6.0 or with 1-ethyl1-3-(3-dimethylaminopropIy)

carbodiimide (EDAC) at concentrations >0.3M, even at neutral pH, resulted in loss of antigenicity of poly α(2→8) NeuNAc (Lifely, M.R., Gilbert, A.S. & Moreno, C. (1981) Carb. Res. 94, 193-201). Accordingly, the CP (5.0 mg/ml in 0.2M NaCl) were derivatized with 0.5M adipic acid dihydrazide (ADH), 0.1M EDAC, pH 6.1 to 7.0 at room temperature for 3 to 4 hr. The pH was maintained in a pH stat with 0.25N HCl. The reaction mixture was dialyzed against 0.2M NaCl at 3-8°C, for 2 days with 3 changes of the outer fluid and passed through 4B-CL Sepharose® in this solvent. The CP-containing fractions were pooled, dialyzed against sterile pyrogen-free water and freeze-dried. The content of adipic acid hydrazide (AH) was assayed by the TNBS reaction (Inman, J.K., & H.M. Dintzis. (1969) Biochem. (USA) 8, 4074-4080; Schneerson, R. et al. (1980) J. Exp. Med. 152, 361-376).

[0020] AH-CP and TT, at equal concentrations of 7.5 to 20 mg/ml in 0.2M NaCl, were adjusted to a pH between 6.1 and 7.0 with 0.1N HCl. Then, 0.1M EDAC was added and this pH maintained at 3-8°C for 3 h. The reaction mixture was dialyzed against 0.2M NaCl at 3-8°C and then passed through 4B-CL Sepharose® in the same solvent. The void volume fractions were pooled, assayed for NeuNAc and protein and stored in 0.01% thimerosal at 3-8°C.

<u>Immunization of Mice</u>. General purpose mice, 4 to 5 weeks old, were injected s.c. with 2.5 µg of NeuNAc in 0.1 ml of saline, either as the CP alone or as the conjugate, 3 times 2 weeks apart (Schneerson, R. et al. (1980) J. Exp. Med. 152, 361-376). Ten mice from each group were exsanguinated 7 days after each injection. None of the mice injected with saline (controls) had antibodies to the CP or to TT (data not shown).

<u>Adsorption</u>. ELISA was used to determine the specificity of IgG poly α(2→8) NeuNAc and poly α(2→9) NeuNAc antibodies. Dilutions of sera that yielded an A in the upper linear part of the curve (1.0 to 1.4) were mixed with 100 µg of either poly α(2→8) NeuNAc, poly α(2→9) NeuNAc, or K92 CP and incubated at 22°C for 2 h and overnight at 3-8°C. Controls were the group A meningococcal and the type III group B streptococcal CP (containing an α (2→8)- linked NeuNAc residue per repeat unit). Adsorption by the CP was calculated as the percent A compared to the unadsorbed sera.

<u>Human sera.</u> Paired maternal and cord sera were donated by James C. Parke Jr, Charlotte Memorial Hospital and Medical Center, Charlotte, NC and Eyal Schiff and Justin Passwell, Sheba Medical Center, Israel.

<u>Statistical Methods.</u> Data analysis was performed using the Statistical Analysis System (SAS). The logarithms of the concentrations were used for all statistical calculations. Antibody concentrations that were below the limit of sensitivity of the ELISA were assigned values equal to one half of that value. Comparison of geometric means was performed with the two-sided t-test and the paired t-test.

<u>EXAMPLE 1</u>

<u>Characterization of the conjugates</u>

[0021] Data of representative conjugates are shown in Table 1. The percent of derivatization of the CP with AH ranged from 0.8 for K1-TT_1 to 10.2 for K92-TT_2. All AH derivatives, except for the latter, yielded an identity reaction with the native CP by double immunodiffusion. The native CP formed a spur over this K92-AH derivative (not shown).

[0022] The protein/NeuNAc ratios were related to the percent derivatization of the CP with AH. K1-TT_1 had the highest protein/NeuNAc ratio (12.8) and contained a CP with 0.8% AH. K92-TT_2, with the lowest ratio (1.4), contained a CP with 10.2% AH. The highest yields of conjugates were obtained when the reaction mixture for conjugation used concentrations of 7.5 to 10 mg/ml of TT and AH-CP.

[0023] All preparations of conjugates eluted at the void volume through CL-4B Sepharose® indicating multipoint attachment between the AH-CP and the TT (Figure 2). Figure 3 provides serologic evidence for the covalent attachment of the CP with the carrier protein (TT). Antiserum to each component precipitated with a line of identity with a representative conjugate, K1-TT_1. Non-identical lines of precipitation were formed when these antisera reacted with mixtures of the CP and TT (not shown).

Table 1.

Characterization of capsular polysaccharide-protein conjugates						
Conjugate	Protein (µg/ml)	NeuNac (µg/ml)	AH/NeuNAc (wt/wt)	Protein/CP ratio	Yield (% CP)	Concentration (mg/ml)*
K1-TT_1**	531	41.4	0.8	12.8	5.0	20
K1-TT_2**	465	96.1	2.6	4.8	9.6	15

* Concentration of the reactants during the conjugation procedure.

** The K1 CP for these conjugates were OAc^-.

Table 1. (continued)

Characterization of capsular polysaccharide-protein conjugates						
Conjugate	Protein (μg/ml)	NeuNac (μg/ml)	AH/NeuNAc (wt/wt)	Protein/CP ratio	Yield (% CP)	Concentration (mg/ml)*
$K1_{OAc+}$-TT***	630	262	1.9	2.4	28.8	10
MenB-TT_1	463	94.5	1.8	4.9	5.0	15
MenB-TT_2	314	51.1	2.3	6.2	4.6	15
K92-TT_1	294	98.8	3.4	3.0	10.5	15
K92-TT_2	705	517	10.2	1.4	51.7	7.5
MenC-TT	234	121	8.5	1.9	22.5	10

* Concentration of the reactants during the conjugation procedure.

*** K1 CP of the OAc^+ variant of Escherichia coli, strain LH.

Example 2

Induction of poly α(2→8) NeuNAc antibodies (Table 2).

[0024] The four CP did not elicit rises of IgM or IgG antibodies. All four α(2→8) NeuNAc conjugates (K1-TT_1, K1-TT_2, MenB-TT_1 and MenB-TT_2) elicited statistically significant rises in IgM antibodies. An IgM booster response was elicited after the second injection by these conjugates; the levels elicited by K1-TT_1 and MenB-TT_1 were higher than those elicited by the other two poly α(2→8) NeuNAc conjugates ($p<0.001$). Only K1-TT_2 and MenB-TT_2 elicited IgM booster responses after the third injection.

[0025] The four poly α(2→8) NeuNAc conjugates elicited statistically significant rises of IgG antibodies after the second and third injections. The IgG levels elicited by the third injection of MenB-TT_2 (4.29 U/ml) were higher than those elicited by the other three conjugates but not significant (NS). One mouse in this group, however, had 240 U/ml and the geometric mean level, excluding this animal, was 2.74 U/ml.

[0026] $K1_{OAc+}$ -TT, prepared from E. coli strain LH, elicited high levels of IgM and IgG antibody to the OAc^+ variant of this CP and low antibody levels to poly α(2→8) NeuNAc.

[0027] The two K92-TT elicited both IgM and IgG poly α(2→8) NeuNAc antibodies; the IgG levels were higher than those elicited by the K1-TT_1 (P=0.01), K1-TT_2 (P=0.0001), MenB-TT_1 (P=0.0002) and MenB-TT2 (p<0.05). K92-TT2, containing the heavily derivated K92 CP, also elicited higher IgG antibody levels than the KI-TT and MenB-TT conjugates.

[0028] MenC-TT did not elicit poly α(2→8) NeuNAc antibodies in any of the mice.

[0029] The specificity of the antibodies was shown by adsorption experiments using sera from mice injected with killed bacteria or by three injections of the conjugates (data not shown). Poly α(2→8) NeuNAc and poly α(2→9) NeuNAc adsorbed homologous IgG antibodies from the antisera (50-89%). The K92 CP adsorbed both poly α(2→8) and poly α(2→9) NeuNAc antibodies (69-89%). The two controls (group A meningococcal and group B type III streptococcal CP) adsorbed <10% of either poly α(2→8) or α(2→9) NeuNAc antibodies.

Table 2.

Serum IgG and IgM antibodies to the capsular polysaccharide of Group B Neisseria meningitidis and Escherichia coli K1 (poly α(2→8) NeuNAc).						
	Post-immunization geometric mean					
	IgM (μg/ml)			IgG (ELISA U)		
Immunogen	1st	2nd	3rd	1st	2nd	3rd
K1	0.09	0.12	0.11[a]	0.05	0.05	0.06[c]
K1-TT_1	0.32	3.35	1.63[b]	0.10	0.49	2.44[d]
K1-TT_2	0.12	0.19	0.62[b]	0.06	0.13	1.95[e]
$K1_{OAc+}$-TT*	0.17	0.16	0.08	0.07	0.20	0.72

b vs a: P<0.001, h vs i: P=0.007, h vs g: P<0.05, h vs f, e:P<005, h vs d: P=0.01

* The second set of values for conjugate $K1_{OAc+}$-TT was determined using OAc^+ K1 CP as the antigen.

Table 2. (continued)

Serum IgG and IgM antibodies to the capsular polysaccharide of Group B Neisseria meningitidis and Escherichia coli K1 (poly α(2→8) NeuNAc).						
	Post-immunization geometric mean					
	IgM (μg/ml)			IgG (ELISA U)		
Immunogen	1st	2nd	3rd	1st	2nd	3rd
	38.7	27.2	7.18	0.16	12.1	56.1
MenB	0.05	0.05	0.05[a]	0.05	0.05	0.05[c]
MenB-TT_1	0.67	1.59	1.50[b]	0.08	0.45	1.81[f]
MenB-TT_2	0.08	0.26	0.72[b]	0.05	0.11	4.29[g]
K92	0.05	0.05	0.05[a]	0.05	0.05	0.05[c]
K92-TT_1	0.09	0.49	1.20[b]	0.05	0.25	17.2[h]
K92-TT_2	0.28	0.78	0.47[b]	0.05	0.83	4.52[i]
MenC	0.05	0.05	0.05	0.05	0.05	0.05
MenC-TT	0.05	0.05	0.05	0.05	0.05	0.05

EXAMPLE 3

Induction of poly α(2→9) NeuNAc and TT Antibodies (Table 3)

[0030] The homologous CP induced low levels of poly α(2→9) NeuNAc IgM antibodies. Neither the homologous nor the heterologous CP induced IgG antibodies.

[0031] All the conjugates elicited IgM antibodies after the first injection. These levels declined after the 2nd and 3rd injections of the MenC-TT and K92-TT conjugates and increased only after the first two injections of the K1-TT conjugates.

[0032] Only the MenC-TT elicited poly α(2→9) NeuNAc IgG antibodies after the first injection; all the conjugates elicited increases after the second and third injections. The highest levels were elicited by MenC-TT > K92-TT > K1-TT. Similar to those observed with poly α(2→8) NeuNAc antibodies, the IgG antibody levels elicited by K92-TT_1 were higher than those elicited by K92-TT_2 but N.S.

[0033] TT antibodies were elicited by all the conjugates with booster responses after each injection similar to those reported for other conjugates using this protein as a carrier (data not shown) (Robbins, J.B. & Schneerson, R. (1990) J. Infect. Dis 161, 821-832; Lagergard, T. et al. (1990) Infect. & Immuno. 58, 687-694).

Table 3.

Serum IgG and IgM antibodies (μg/ml) to the capsular polysaccharide of group C Neisseria meningitidis (poly α (2→9) NeuNac)						
	IgM			IgG		
Antigen	1st	2nd	3rd	1st	2nd	3rd
K1	0.05	0.05	0.05[a]	0.05	0.05	0.05[e]
K1-TT_1	0.11	0.32	0.14[b]	0.05	0.14	1.2[f]
K1-TT_2	0.23	1.09	0.40[b]	0.05	0.97	3.32[f]
MenC	0.05	0.08	0.11[c]	0.07	0.10	0.05[e]
MenC-TT	2.26	0.89	0.53[d]	1.87	18.4	107.5[g]
K92	0.05	0.05	0.05[a]	0.05	0.05	0.05[e]
K92-TT_1	3.23	2.85	0.68[b]	0.05	0.70	21.4[h]
K92-TT_2	1.87	0.74	0.15[b]	0.06	1.71	15.9[h]
b vs a: P=0.0001, d, vs c: P=0.0004, c vs a: P<0.001, f,g,h, vs e: P=0.0001, g vs f,h: P<0.001						

EXAMPLE 4

Temperature-dependent Binding of IgG Antibodies (Table 4)

[0034] Binding to the two CP by IgG antibodies elicited by K92-TT, K1-TT and MenC-TT conjugates and E. coli K92 and M. nonliquefaciens cells was assayed at 22°C and at 37°C. Reduction in binding at 37°C of poly α(2→8) NeuNAc antibodies elicited by the K1-TT_2, M. nonliquefaciens, and K92-TT_1 was similar (~40%). In contrast, there was only ≤10% reduction in binding of poly α(2→9) NeuNAc antibodies elicited by K1-TT_2, K92-TT_1, MenC-TT and E. coli K92 cells. These data are consistent with other results (Mandrell, R.E. & Zollinger, W.D. (1982) J. Immunol. 129, 2172-2178).

Table 4.

Temperature-dependent binding of murine poly α(2→8) and poly α(2→9) NeuNAc IgG antibodies (percent binding at 37°C compared to 22°C)		
	CP used for ELISA	
Immunogen	Poly α(2→8) NeuNAc	poly α(2→9) NeuNAc
K1-TT_2	41.8%	90.9%
M. nonliquefaciens cells	34.6	N.D*
K92-TT_1	49.1%	93.8%
E.coli K92 cells	N.D.	91.5%
MenC-TT	N.D.	100%

*N.D. Not detectable

EXAMPLE 5

Poly α(2→8) NeuNAc antibodies in paired maternal and cord sera (Table 5)

[0035] Most women at term had detectable IgM and IgG poly α(2→8) NeuNAc antibodies. The IgM and IgG levels of the Israeli women were higher than those of the women in Charlotte, NC (P+0.0001). As expected, the IgM poly α (2→8) NeuNAc antibodies in the cord were at trace or non-detectable levels. The GM levels of IgG antibodies in the cord sera were significantly higher than those of the mothers from both regions. Most of the cord poly α(2→8) NeuNAc IgG antibodies were higher than those of the corresponding maternal sera (69/81).

Table 5.

IgG and IgM antibodies to poly α(2→8) NeuNAc in paired human mother-newborn (umbilical cord) sera (Geometric mean)						
		Maternal		Cord		Maternal IgG
Source	*n*	IgM	IgG	IgM	IgG	vs cord IgG
Charlotte, NC	36	0.35	26.9	0.03	32.9	P=0.003
Sheba Medical Center, Israel	45	0.91	80.0	0.04	121	P=0.0001
The levels of IgM antibodies are expressed as µg Ab/ml and the levels of IgG antibodies as percent of a high-tittered adult serum (GH) as ELISA units.						

EXAMPLE 6

Passive Immunization

[0036] Either monoclonal or polyclonal antibodies, of human or animal origin, for passive immunization for prevention, or as adjunct therapy of systemic infections with organisms containing poly α(2→8) NeuNAc or poly α(2→9) NeuNAc surface antigens in an animal, including humans, may be produced by the above-described conjugate vaccines. Passive immunization, for both therapeutic and preventative purposes, has been carried out since the turn of the century. Passive immunization has been considered again for prevention of group B meningococcus systemic infections in-

cluding meningitis, as well as other capsulated bacterial pathogens that cause systemic infections including the pneumococcus, Hemophilus influenza type b, group B streptococcus and E. coli infections in hosts at higher risk than the general population including fetuses, newborns and patients with congenital or acquired immunodeficiencies (Patients with immunodeficiencies may not be capable of producing protective levels of antibodies when injected with K92 and/or poly $\alpha(2\rightarrow8)$ NeuNAc conjugate vaccines). The technique of passive immunization is taught by: Flexner (1913) J. Exp. Med. 17:553-570; Brahahm (1938) Proc. Soc. Exp. Biol. Med. 30:348; Raff et al. (1988) J. Infect. Dis. 157:118-126; Kim et al. (1985) Infect. Immuno. 50:734-737; and Latson et al. (1988) Podiatr. Infect. Dis. 7:747-752.

EXAMPLE 7

Active Immunization Against the Three Major Serogroups of N. meningitidis

[0037] Active immunization against the three major serogroups of Neisseria meninaitidis, would include conjugate vaccines of group A along with the conjugate vaccines described-above. A trivalent polysaccharide-protein conjugate vaccine, capable of eliciting serum antibodies to groups A, B, and C meningococcal meningitis and thereby preventing most of the systemic infections, including meningitis, caused by Neisseria meningitidis, may be produced by this method using the above-described conjugates and a group A meningococcal conjugate. Group A meningococcal polysaccharide protein conjugates have been synthesized according to a published method (Chu et al. (1983) Infect. Immun. 40: 245-256). Concurrent injection of more than one polysaccharide protein conjugate in animals and in humans has been shown to elicit protective levels of antibodies to each component and at equal levels as those elicited by each conjugate injected separately (Schneerson et al. (1986) Infect. Immun. 52:501-518).

Claims

1. The use of a conjugate of *Escherichia coli* K92 capsular polysaccharide and a carrier protein, for the manufacture of a medicament for use in the prevention of infection caused by non-K92 bacterial microorganisms having poly $\alpha(2\rightarrow8)$NeuNAc capsular polysaccharide surface antigens and by non-K92 bacterial microorganisms having poly $\alpha(2\rightarrow9)$NeuNAc capsular polysaccharide surface antigens, wherein the conjugate elicits the production of antisera containing (i) antibodies reactive with the non-K92 bacterial microorganisms having poly $\alpha(2\rightarrow8)$NeuNAc capsular polysaccharide surface antigens; (ii) antibodies reactive with the non-K92 bacterial microorganisms having poly $\alpha(2\rightarrow9)$NeuNAc capsular polysaccharide surface antigens; and (iii) antibodies reactive with the carrier protein.

2. Use of antisera elicited by a conjugate of *Escherichia coli* K92 capsular polysaccharide and a carrier protein and containing (i) antibodies reactive with non-K92 bacterial microorganisms having poly $\alpha(2\rightarrow8)$NeuNAc capsular polysaccharide surface antigens; (ii) antibodies reactive with the non-K92 bacterial microorganisms having poly $\alpha(2\rightarrow9)$NeuNAc capsular polysaccharide surface antigens; and (iii) antibodies reactive with the carrier protein; for the manufacture of a medicament for use in passive immunisation against infection by non-K92 bacterial microorganisms having poly $\alpha(2\rightarrow8)$NeuNAc capsular polysaccharide surface antigens and by non-K92 bacterial microorganisms having poly $\alpha(2\rightarrow9)$NeuNAc capsular polysaccharide surface antigens.

3. Use according to claim 2, wherein the antisera contain antibodies selected from the IgM and IgG classes.

4. Use according to claim 1, wherein the medicament is a vaccine.

5. Use according to any preceding claim, wherein (i) the antibodies are reactive with *Escherichia coli* K1 or Group B *N. meningitidis* having poly $\alpha(2\rightarrow8)$NeuNAc polysaccharide surface antigens, and (ii) with the Group C *N. meningitidis* having poly $\alpha(2\rightarrow9)$NeuNAc polysaccharide surface antigens.

6. Use according to any preceding claim, wherein the carrier protein is immunogenic and is selected from albumin, detoxified diphtheria toxin, tetanus toxoid, detoxified exotoxin A of *Pseudomonas aeruginosa*, detoxified *Staphylococcus aureus* toxin, synthetic polypeptides, bacterial outer membrane proteins, and viral proteins.

7. Use according to claim 6, wherein the carrier protein is tetanus toxoid.

8. Use according to any preceding claim, wherein the conjugate is obtainable by the covalent coupling of the capsular polysaccharide and the carrier protein with a linker selected from dihydrazide compounds, diamino compounds, amino-e-caproic acid, and N-hydroxysuccinimide acid anhydride-based heterobifunctional molecules.

9. Use according to claim 8, wherein the linker is a dihydrazide compound.

10. Use according to claim 9, wherein the linker is adipic acid dihydrazide.

11. Use according to claim 8, wherein the linker is diaminohexane.

12. Use according to claim 8, wherein the linker is N-succinimidyl 3-(2-pyridyldithio)propionate.

13. Use according to any preceding claim, for preventing infection by *N. meningiditis* Group B microorganisms.

14. Use aocording to any of claims 1 to 12, for preventing infection by *E. coli* K1 microorganisms.

Patentansprüche

1. Verwendung eines Konjugats aus Escherichia coli K92-Kapselpolysacharid und einem Trägerprotein für die Herstellung eines Medikaments zur Verwendung in der Vorbeugung einer Infektion, die durch Nicht-K92 bakterielle Mikroorganismen mit Poly $\alpha(2\rightarrow8)$-NeuNAc-Kapselpolysacharid-Oberflächenantigenen und durch Nicht-K92 bakterielle Mikroorganismen mit Poly $\alpha(2\rightarrow9)$-NeuNAc-Kapselpolysacharid-Oberflächenantigenen verursacht wird, wobei das Konjugat die Produktion von Antiseren anregt, enthaltend (i) Antikörper, die mit den Nicht-K92 bakteriellen Mikroorganismen mit Poly $\alpha(2\rightarrow8)$-NeuNAc-Kapselpolysacharid-Oberflächenantigenen reagieren, (ii) Antikörper, die mit den Nicht-K92 bakteriellen Mikroorganismen mit Poly $\alpha(2\rightarrow9)$-NeuNAc-Kapselpolysacharid-Oberflächenantigenen reagieren und (iii) Antikörper, die mit dem Trägerprotein reagieren.

2. Verwendung von Antiseren, die durch ein Konjugat aus Escherichia coli K92-Kapselpolysacharid und einem Trägerprotein angeregt werden, und enthalten: (i) Antikörper, die mit Nicht-K92 bakteriellen Mikroorganismen mit Poly $\alpha(2\rightarrow8)$-Neu NAc-Kapselpolysacharid-Oberflächenantigenen reagieren, (ii) Antikörper, die mit den Nicht-K92 bakteriellen Mikroorganismen mit Poly $\alpha(2\rightarrow9)$ NeuNAc-Kapselpolysacharid-Oberflächenantigenen reagieren und (iii) Antikörper, die mit dem Trägerprotein reagieren, für die Herstellung eines Medikaments zur Verwendung in der passiven Immunisierung gegen eine Infektion durch Nicht-K92 bakterielle Mikroorganismen mit Poly $\alpha(2\rightarrow8)$-NeuNAc-Kapselpolysacharid-Oberflächenantigenen und durch Nicht-K92 bakterielle Mikroorganismen mit Poly α $(2\rightarrow9)$-NeuNAc-Kapselpolysacharid-Oberflächenantigenen.

3. Verwendung nach Anspruch 2, wobei die Antiseren Antikörper enthalten, die aus den IgM-und IgG-Klassen ausgewählt sind.

4. Verwendung nach Anspruch 1, wobei das Medikament ein Impfstoff ist.

5. Verwendung nach einem der vorhergehenden Ansprüche, wobei (i) die Antikörper reaktiv sind mit Escherichia coli K1 oder Gruppe B-N. meningitidis mit Poly $\alpha(2\rightarrow8)$ NeuNAc-Polysacharid-Oberflächenantigenen, und (ii) mit der Gruppe C-N. meningitidis mit Poly $\alpha(2\rightarrow9)$-NeuNAc-PolysacharidOberflächenantigenen.

6. Verwendung nach einem der vorhergehenden Ansprüche, wobei das Trägerprotein immunogen ist und ausgewählt ist aus Albumin, entgiftetem Diphterietoxin, Tetanustoxoid, entgiftetem Exotoxin A von Pseudomonas aeruginosa, entgiftetem Staphylococcus aureus-Toxin, synthetischen Polypeptiden, bakteriellen Außenmembranproteinen und viralen Proteinen.

7. Verwendung nach Anspruch 6, wobei das Trägerprotein Tetanustoxoid ist.

8. Verwendung nach einem der vorhergehenden Ansprüche, wobei das Konjugat erhältlich ist durch kovalentes Kuppeln des Kapselpolysacharids und des Trägerproteins mit einem Linker, ausgewählt aus Dihydrazidverbindungen, Diaminoverbindungen, Amino-ϵ-capronsäure und N-Hydroxysuccinimidsäureanhydrid-basierenden heterobifunktionellen Molekülen.

9. Verwendung nach Anspruch 8, wobei der Linker eine Dihydrazidverbindung ist.

10. Verwendung nach Anspruch 9, wobei der Linker Adipinsäuredihydrazid ist.

11. Verwendung nach Anspruch 8, wobei der Linker Diaminohexan ist.

12. Verwendung nach Anspruch 8, wobei der Linker N-Succinimidyl-3-(2-pyridyldithio) propionat ist.

13. Verwendung nach einem der vorhergehenden Ansprüche zur Vorbeugung einer Infektion durch N.meningitidis-Gruppe B-Mikroorganismen.

14. Verwendung nach einem der Ansprüche 1 bis 12 zur Vorbeugung einer Infektion durch E. coli K1-Mikroorganismen.

Revendications

1. Utilisation d'un conjugué de polysaccharide de la capsule d'Escherichia coli K92 et d'une protéine porteuse, pour la fabrication d'un médicament destiné à être utilisé dans la prévention d'une infection causée par des microorganismes bactériens non-K92 ayant des antigènes de surface de polysaccharide de la capsule poly α (2→8) NeuNAc, et par des microorganismes bactériens non-K92 ayant des antigènes de surface de polysaccharide de la capsule poly α(2→9) NeuNAc dans laquelle le conjugué provoque la production d'antisérums contenant (i) des anticorps réagissant avec les microorganismes bactériens anti-K92 ayant des antigènes de surface de polysaccharide de la capsule poly α(2→8) NeuNAc ; (ii) des anticorps réagissant avec les microorganismes bactériens non-K92 ayant des antigènes de surface de polysaccharide de la capsule poly α (2→9) NeuNAc ; et (iii) des anticorps réagissant avec la protéine porteuse.

2. Utilisation d'antisérums produits par un conjugué de polysaccharide de la capsule *d'Escherichia coli* K92 et d'une protéine porteuse et contenant (i) des anticorps réagissant avec des microorganismes bactériens non-K92 ayant des antigènes de surface de polysaccharide de la capsule poly α(2→8)NeuNAc ; (ii) des anticorps réagissant avec les microorganismes bactériens non-K92 ayant des antigènes de surface de polysaccharide de la capsule poly α (2→9)NeuNAc ; et (iii) des anticorps réagissant avec la protéine porteuse ; pour la fabrication d'un médicament destiné à être utilisé dans l'immunisation passive contre une infection par des microorganismes bactériens non-K92 ayant des antigènes de surface de polysaccharide de la capsule poly α(2→8)NeuNAc et par des microorganismes bactériens non-K92 ayant des antigènes de surface de polysaccharide de la capsule poly α(2→9)NeuNAc.

3. Utilisation selon la revendication 2, dans laquelle les antisérums contiennent des anticorps sélectionnés parmi les classes d'IgM et d'IgG.

4. Utilisation selon la revendication 1, dans laquelle le médicament est un vaccin.

5. Utilisation selon l'une des revendications précédentes, selon laquelle (i) les anticorps réagissent avec *Escherichia coli* K1 ou *N. meningitidis* du group B ayant des antigènes de surface de polysaccharide de la capsule poly α(2→8) NeuNAc, et (ii) avec les antigènes de *N. meningitidis* du group C ayant des antigènes de surface de polysaccharide de poly α(2→9)NeuNAc.

6. Utilisation selon l'une des revendications précédentes, dans laquelle la protéine porteuse est immunogénique et est choisie parmi l'albumine, la toxine de la diphtérie détoxifiée, l'anatoxine tétanique, l'exotoxine A détoxifiée de *Pseudomonas aeruginosa,* la toxine détoxifiée du *Staphylococcus aureus,* les polypeptides synthétiques, les protéines de la membrane externe bactérienne, et des protéines virales.

7. Utilisation selon la revendication 6, dans laquelle la protéine porteuse est l'anatoxine tétanique.

8. Utilisation selon l'une des revendications précédentes, dans laquelle le conjugué est obtenu par un couplage covalent de polysaccharide de la capsule et de la protéine porteuse avec un lien choisi parmi les composés dihydrazide, diamino, acide amino-ε-caproique, et des molécules hétérobifonctionnelles à base d'arhydrido d'acide N-hydroxysuccinimide-anhydride.

9. Utilisation selon la revendication 8, dans laquelle le lien est un composé de dihydrazide.

10. Utilisation selon la revendication 9, dans laquelle le lien est l'acide dihydrazide adipique.

11. Utilisation selon la revendication 8, dans laquelle le lien est le diaminohexane.

12. Utilisation selon la revendication 8, dans laquelle le lien est le propionate de succinidyl 3-(2-pyrijdyldithio).

13. Utilisation selon l'une des revendication précédentes, pour prévenir des infections par des microorganismes *N. meningiditis* du groupe B.

14. Utilisation selon l'une des revendications 1 à 12, pour prévenir des infections par des microorganismes *E. coli* K1.

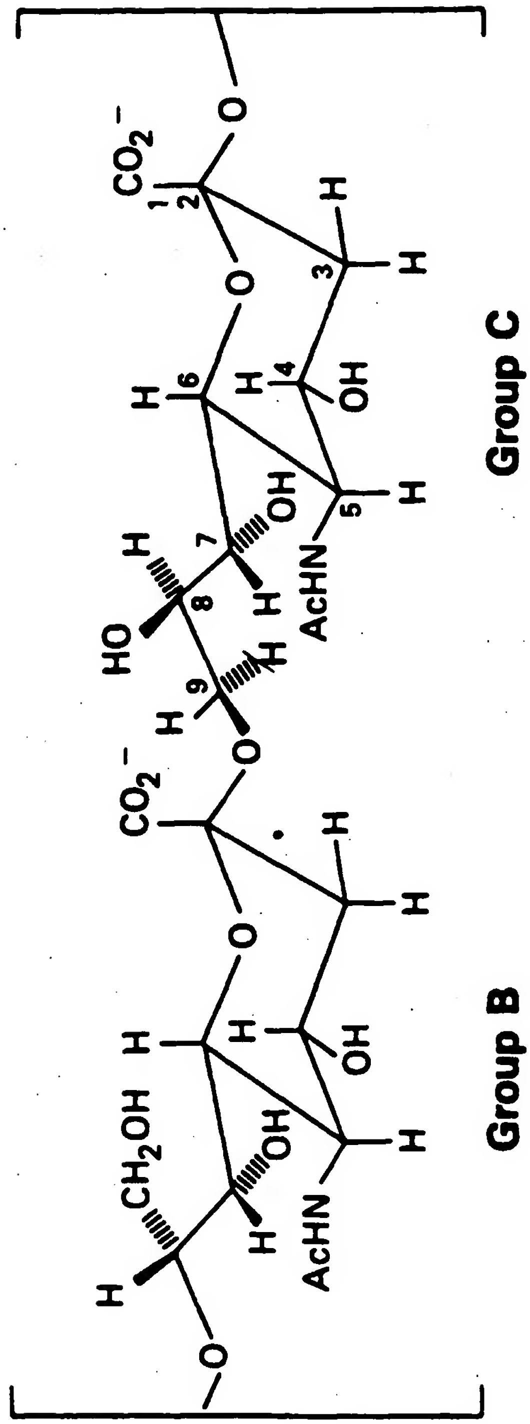

FIG. I

FIG. 2

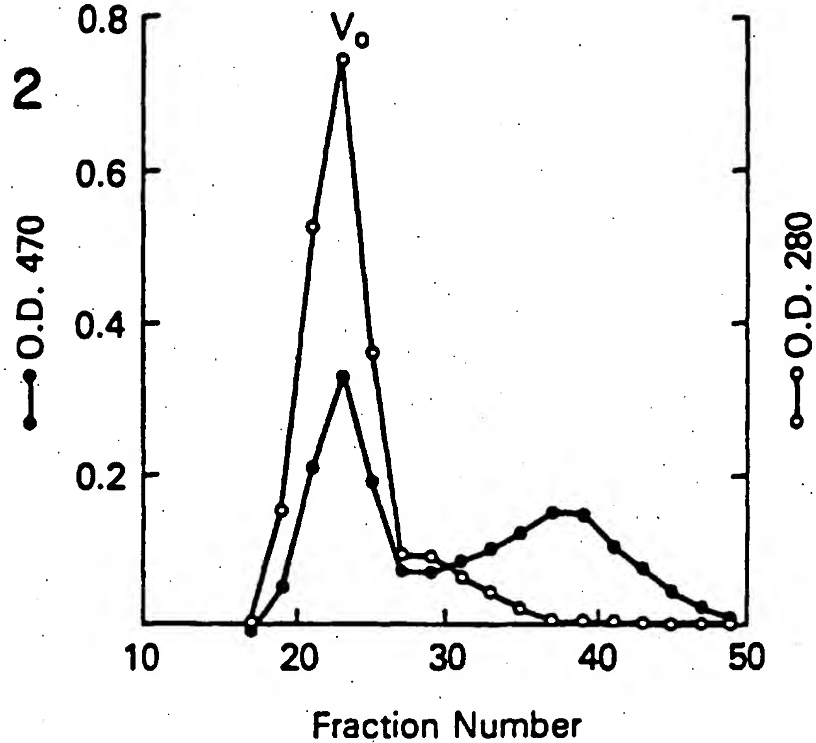

FIG. 3